

**STRAIN SPECIFIC BEHAVIOURAL RESPONSE TO ENVIRONMENTAL  
ENRICHMENT IN THE MOUSE**

HA Van de Weerd<sup>1</sup>, V Baumans<sup>1</sup>, JM Koolhaas<sup>2</sup> and LFM Van Zutphen<sup>1</sup>

<sup>1</sup>*Department of Laboratory Animal Science, Utrecht University*

<sup>2</sup>*Department of Animal Physiology, University of Groningen*

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### SUMMARY

*The influence of environmental enrichment on the behaviour of the mouse has been studied in two inbred strains (C57BL and BALB/c). Male mice of each of the two strains were subjected to behavioural tests after being housed for two months either under standard housing conditions or in an enriched environment. The results of the behavioural tests indicated that the C57BL mice housed in the enriched environment were more reactive and alert compared to mice housed in the standard environment. In the BALB/c mice results may be interpreted as if enriched environments lead to an increased level of anxiety. It is concluded that environmental enrichment has a strain specific effect on the behaviour of mice.*

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## INTRODUCTION

Most environments of laboratory animals have been designed to serve human convenience, with little or no consideration for the animal's nature. Laboratory housing conditions deprive the animal of the possibility of performing its full repertoire of normal behaviour. As a response to this lack of stimulation the animal may show stereotyped behaviour or passiveness (Wemelsfelder 1990).

Environmental enrichment (i.e. additions to an animal's environment with which it can interact) may improve the well-being of captive animals (Beaver 1989). According to Chamove (1989a) the objective of environmental enrichment is to alter behaviour so that it is within the range of the animal's normal behaviour.

A large body of literature has been published on ways to improve the environment of captive animals, e.g. by providing animals with toys or other cage accessories (Bayne et al 1992; Brooks et al 1993; Scharmann 1991). Less attention has been paid to the evaluation of the enrichment program being used (Bloomsmith et al 1991). To obtain a complete picture of the impact of an environmental change on an animal, both frequency and duration of behavioural changes can be determined (Bayne et al 1993). Furthermore, physiological variables, such as heart rate, hormonal levels or reproductive function, can be monitored to assess the responses to changes in laboratory environments (Markowitz & Line 1990). Another method of evaluating environmental enrichment is to observe if the enrichment has effects on the behaviour of animals in behavioural tests (Manosevitz 1970).

In those studies which do evaluate the behavioural effects of enrichment, rats or larger laboratory animals have been monitored (e.g. Holson 1986) but only few authors have studied these effects in mice (Manosevitz 1970; Manosevitz & Montemayor 1972). The aim of our study was to employ behavioural tests to evaluate the behaviour of mice from standard or enriched environments and to investigate if the enrichment had different effects on the behaviour of different strains.

Three tests were used to compare the behaviour of mice of the C57BL and BALB/c strains, housed in standard or enriched environments. The tests were chosen because they deal with different aspects of behaviour. They can also give an indication of the degree of alertness or anxiety, which can possibly alter as a consequence of different housing conditions. The hole board test has first been described by Boissier & Simon (1962) and is designed to test exploratory behaviour, as it takes advantage of the natural tendency of mice to dip their heads into holes. In the cage emergence test a mouse is placed into an unfamiliar cage and the reactivity to escape from this novel environment is measured. An open field test was used to compare the behaviour of the animals in this novel environment. In the second part of the test, the reaction of the animals to a sudden change in the

acoustic environment was studied in order to reveal whether or not the mice are responsive to novel environmental stimuli.

## ANIMALS AND METHODS

### *Animals*

Thirty-two male mice from two inbred strains, C57BL/6JlcoU (n=16) and BALB/c AnCrRyCpbRivU (n=16) were used. Animals were reared by individual mothers in an SPF environment. Nesting material (cotton or tissues) was provided to enhance breeding. When the experiment started they were at the age of three weeks. The mice were housed and maintained in a room with conventional hygiene and controlled photo-period (6.00-18.00 h, white light 225 lux), relative humidity (62-66%), temperature (22-23 °C) and ventilation (15 air changes per h). Tap water and food pellets were provided ad libitum (food pellets RMH-B, Hope Farms, Woerden, The Netherlands).

### *Housing*

Per strain, a group of eight animals was housed in a wire-topped Macrolon type III cage (840 cm<sup>2</sup>, UNO roestvaststaal, Zevenaar, The Netherlands) either under enriched conditions or under standard laboratory conditions.

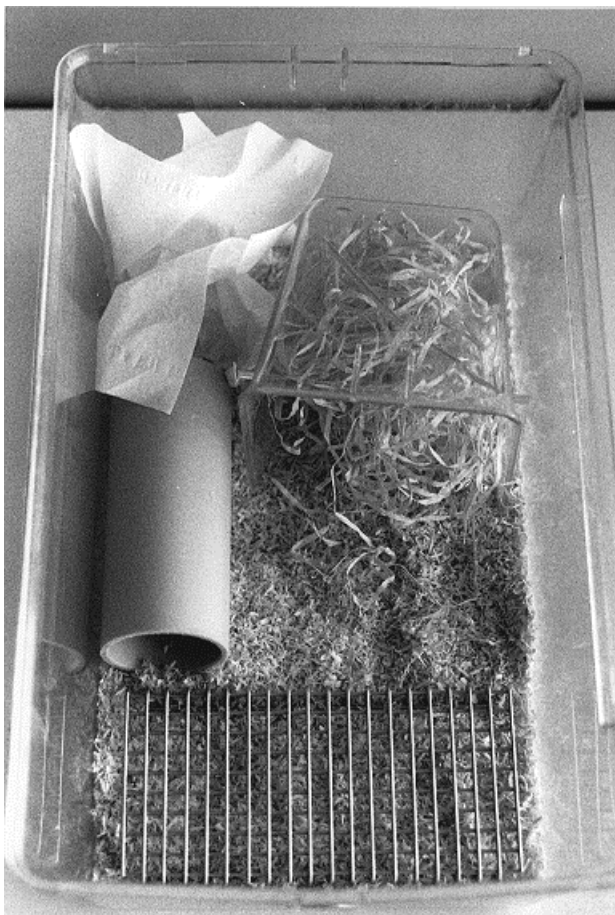
In the enriched environment the cages were provided with 125 g of sawdust (Lignocel 3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany) and a set of well-defined objects. These objects were: a nest box, consisting of one half of a Macrolon type I cage (204 cm<sup>2</sup>) placed on its side, a grid floor (19x10 cm, stainless steel wire, mesh size 10x10 mm<sup>2</sup>) placed beneath the food hopper on the bedding, and an opaque plastic tube (Ø 6 cm, length: 16 cm). Additionally nesting material was provided in the form of three tissues (Kleenex, Kimberley-Clark) and 10 g of wood-wool (BMI, Helmond, The Netherlands). The tissues and wood-wool were renewed after cage cleaning once a week. The location of the objects in the enriched cage remained the same during the experiment (Figure 1). In the standard environment the cages were provided only with 125 g of sawdust.

### *Behavioural testing*

During the study, which lasted 2 months, the animals were subjected individually to the three behavioural tests.

*Hole board test.* A mouse was placed in the centre of a plastic board, measuring 37.5x37.5x3.5 cm, pierced with 16 holes (Ø 3 cm) in 4 rows. The board was fixed at

a distance of 15 cm above a table, light intensity during testing was 270-300 lux (board level). The board was covered with a transparent lid (40x40x20 cm) to prevent the mice from remaining at the edge of the board.



**Figure 1** *The location of the objects in the enriched cage.*

The number of holes explored by a mouse during 3 min of testing was counted. A dip was registered if a mouse put its head in a hole at least up to the eye level. Repeated dips into the same hole were not counted unless these were separated by locomotion. Afterwards faeces production was registered. The mice were subjected to this test twice during this study, for the first time on day 29 (enriched) or 30 (standard) and for the second time on day 64 (enriched) or 65 (standard). Testing was performed between 14.00 and 15.00 h.

*Cage emergence test.* The apparatus consisted of a Macrolon type I cage (204

cm<sup>2</sup>) with a hole (Ø 4 cm) in one of the side walls. There was no lid on the cage. Light intensity during testing was 350 lux (floor level). A mouse was placed inside the cage with its back to the opening and the time to escape from the cage (all four feet outside the cage) was registered. The maximum testing time was set at 10 min. Afterwards the number of faecal boli was counted. The mice were subjected to this test between 10.30 and 11.30 h on day 43 (enriched) or at 14.30 h on day 44 (standard).

*The open field test* consisted of a circular, transparent pvc floor circle (Ø 90 cm) surrounded by an opaque pvc wall (height: 50 cm). Light intensity during testing was 25 lux (floor level). The test was combined with a sudden silence test. During the first part of the open-field test white noise produced by a random-noise generator (General Motor Company) was present (75 dB). After 4 min of behavioural observation this noise was suddenly turned off (remaining background noise: 45 dB) and the behaviour of the mouse was scored for another 4 min.

Each test was recorded with a camera-videosystem, the experimenter not being present in the testing room. Afterwards the behaviour of the animals was scored from the videotape (software used: the Observer v 2.0, Noldus b.v. Wageningen, The Netherlands). The following behavioural elements were recorded during both parts of the open-field test:

Time spent on:

- locomotion = movement of the whole body
- immobility = non-locomotion, head movements were allowed
- grooming = scratching, wiping or licking fur, head or tail
- freezing = motionless, no movements of head or body, attentive

Frequency of:

- rearing = upright posture standing on hind feet, including leaning against the wall

The number of faecal boli were counted after each test. The mice were subjected to the open-field test between 13.00 and 15.00 h on day 58 (enriched) or 59 (standard). All the tests apparatuses were cleaned with ethanol (70 %) after an animal was being tested.

During the study the groups of mice were observed daily in their home cage (sleeping site, social interactions/fighting, responses to enrichment objects). Food intake per group was measured weekly and body weight was measured at the beginning of the study, after one month and after two months, near the end of the study.

### *Statistics*

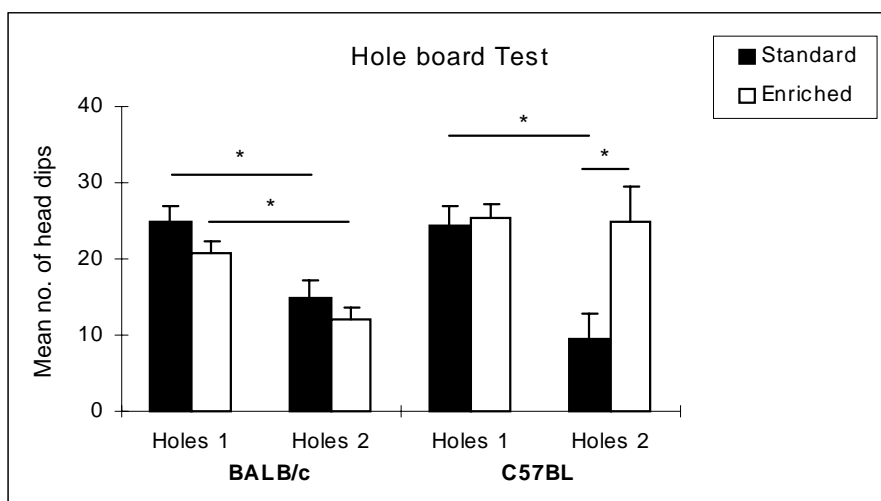
Data are given as mean values  $\pm$  SEM. The results of the tests were analysed by using a SPSS/PC+ statistical computer program (SPSS Inc. Chicago, USA).

The hole board test results were analysed for differences between housing conditions and differences in time by ANOVA followed by t-tests. The results of the cage emergence test were analysed per strain for differences between housing conditions with a Mann Whitney U test. The registered behavioural elements of the open field test were statistically analysed using MANOVA, with the behavioural elements as dependent variables and noise as a within subjects factor, to evaluate differences between the animals of the two housing conditions. Furthermore food intake was analysed by a paired t-test to compare food intake of the groups per date, body weight was analysed by ANOVA. For all the tests the level of statistical significance was pre-set at  $P < 0.05$

## RESULTS

The results of the first hole board test (Figure 2) showed that after one month no significant differences between the animals housed under enriched or standard conditions could be demonstrated in number of holes explored. When the animals were subjected to this test for the second time the C57BL mice housed in the enriched environment explored significantly more holes than the animals housed in a standard cage ( $P < 0.05$ ).

When comparing the results of both hole board tests, it appeared that the BALB/c strain showed a significant decrease in number of holes explored in both groups of mice ( $P < 0.01$ ). In the C57BL strain mice from the standard environment also showed a significant decrease in number of holes explored ( $P < 0.01$ ), but the animals from the enriched environment retained the high exploration level of the first test. In the BALB/c strain there were no significant differences in faeces production between mice from the two housing conditions in both tests, nor in faeces production between the two tests. The C57BL mice did not produce any faeces at all.



**Figure 2** Effects of housing conditions on the behavioural responses of male mice in the hole board test. Holes 1 = first hole board test, Holes 2 = second hole board test. Data are expressed as mean numbers of head dips  $\pm$  SEM, N=32. ANOVA and t-tests were used to calculate statistical differences. \*  $P < 0.05$

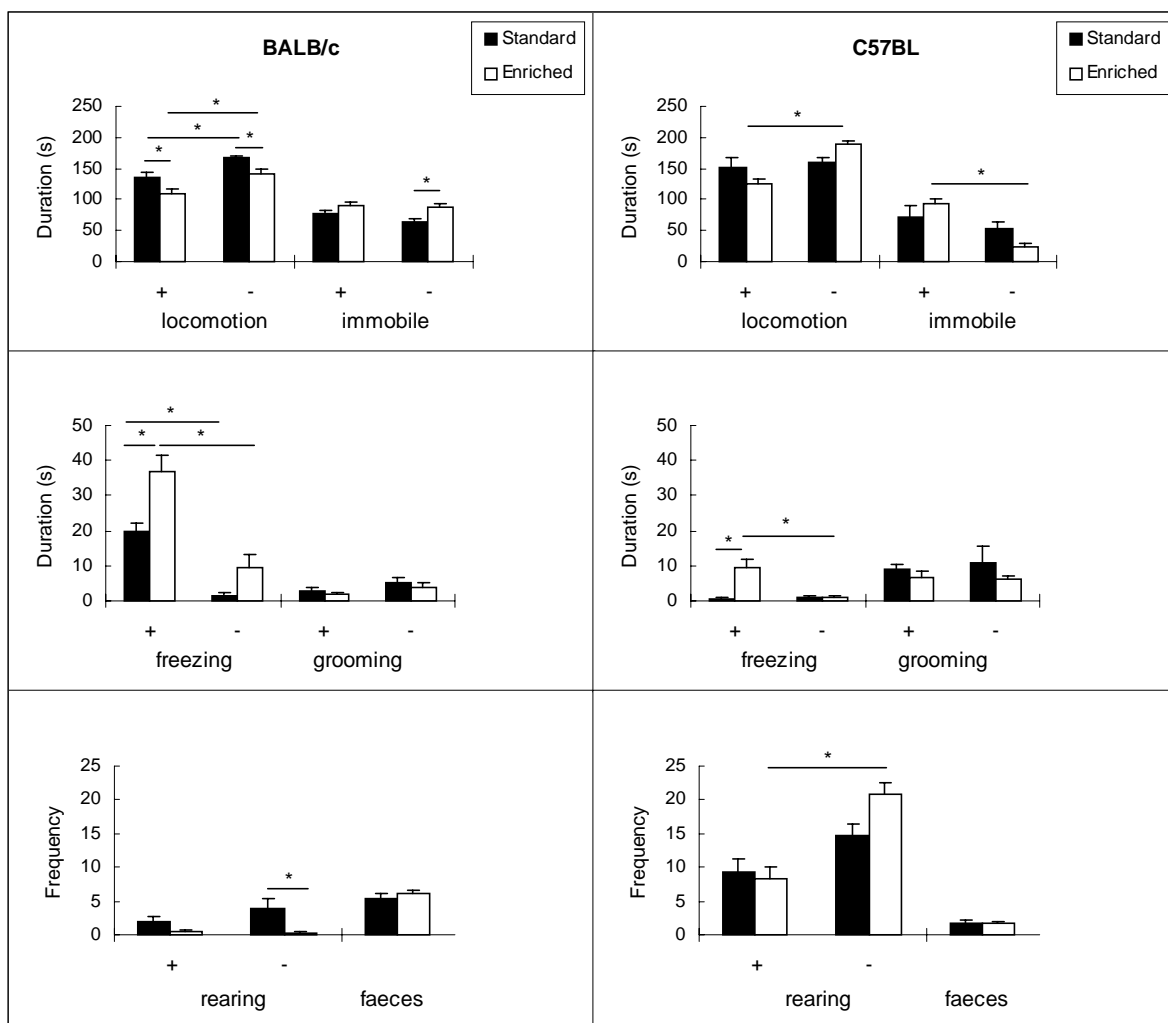
In the cage emergence test (Table 1) the C57BL mice from the enriched environment escaped from the cage significantly faster than the standard housed animals ( $P < 0.01$ ). The BALB/c mice housed under different conditions did not differ significantly from each other. There were no significant differences in faeces production between the two groups of BALB/c mice. The C57BL mice did not produce faeces during the test.

**Table 1** Mean time (s  $\pm$  SEM) to escape from a cage in male mice from two housing conditions (N=32)

	Standard	Enriched	P-value
C57BL	16.1 $\pm$ 2.3	6.9 $\pm$ 1.0	$P < 0.01$
BALB/c	88.1 $\pm$ 73.2	223.1 $\pm$ 100.2	ns

Differences between housing conditions analysed with Mann Whitney U test.

The results of the open field test are presented graphically in Figure 3. Significant differences between animals from the two housing conditions could be demonstrated in a number of behavioural parameters. In the BALB/c strain the enriched housed animals when compared to the standard housed animals, spent less time on locomotion during the first part of the test with noise on ( $P < 0.01$ ) and during the second part of the test without noise ( $P < 0.01$ ). They showed more freezing behaviour during the first part of the test ( $P < 0.01$ ) and more immobility ( $P < 0.05$ ) and less rearing ( $P < 0.05$ ) during the second part of the test.



**Figure 3** Effects of housing conditions on the behavioural response of male mice in the open field test combined with a sudden silence test. + indicates that noise is present and - indicates that noise is absent. Data are expressed as mean duration and frequency  $\pm$  SEM,  $N=32$ . MANOVA and  $t$ -tests were used to calculate statistical differences. \*  $P<0.05$

In the C57BL strain, mice from the enriched environment showed significantly more freezing behaviour during the first part of the test ( $P<0.01$ ) compared to standard housed animals. In both strains there were no differences in number of faecal boli produced by animals from both housing conditions.

The behavioural reaction of the animals from both housing conditions to the sudden change in acoustic conditions, was studied by comparing the time spent on a behavioural element in the first part of the test with the time spent on this element in the second part of the test.

In the second part of the test, mice from the BALB/c strain spent significantly less time on locomotion and freezing compared to the first part of the test. This was observed in animals from both housing conditions (for both groups: locomotion

$P < 0.01$ , freezing  $P < 0.01$ ). In the second part of the test C57BL mice from the enriched cage spent more time on locomotion ( $P < 0.01$ ) and rearing ( $P < 0.01$ ) and less time on immobility ( $P < 0.01$ ) and freezing ( $P < 0.01$ ). These effects were not seen in animals housed in the standard environment.

When comparing both strains the behavioural tests revealed differences between mice housed in the two environments, but these effects were not the same in the two strains. In the open field test the two groups of BALB/c mice differed in the time spent on several behavioural elements, whereas the two groups of C57BL mice mainly differed in the way their behaviour has changed in the second part of the test compared to the first part. The hole-board test and cage emergence test revealed differences only between the two groups of C57BL mice, the animals from the enriched conditions explored more and escaped from the cage faster.

Differences between the strains were also present in the way they responded to the enrichment objects, according to observations in the animal room. For example the C57BL mice slept mostly in the nest box and underneath the foodhopper in the tissues, whereas the BALB/c mice preferred to sleep in the nest box and in the plastic tube. Furthermore the BALB/c mice soiled the enrichment objects with urine and faeces, whereas the C57BL mice did not.

In both strains a remarkable difference between the two housing conditions was observed in sleeping behaviour. Animals in the standard environment always slept together in one group on top of each other, whereas the animals in the enriched environment slept mostly in two or three smaller groups. Furthermore the enriched housed groups had certain locations in their cage where they urinated mostly. The animals in the standard cages seemed to urinate randomly on the bedding.

Sometimes some fighting occurred, but although this behaviour was not quantified it happened more often in the standard groups than in the enriched groups. Especially in the BALB/c strain, in both environments fighting increased gradually when the animals grew older.

In both strains the mice from the two housing conditions did not differ in body weight. No differences were found in weekly food intake between the two groups of BALB/c mice. The amount of food consumed by the C57BL enriched animals was significantly less ( $P < 0.05$ ) than the amount of food consumed by the standard housed animals.

## DISCUSSION

The results of the first hole board test did not indicate differences between mice from the two housing conditions in both strains. In the second hole board test a decrease in number of holes explored was found in all groups except the C57BL enriched animals. Dorr et al (1971) performed a comparable hole board test and repeated this test after one week. They also found a reduction in number of head dips into the holes. The mice were less active and more hesitant, they sniffed more and walked less deliberately. The authors regarded this as a sign of reduced curiosity or habituation. It might indicate that explorative behaviour is diminishing with time. This decrease might be prevented or perhaps postponed with an enriched environment in the C57BL mice but not in the BALB/c mice.

In the emergence test, the C57BL enriched housed animals escaped from the cage significantly faster than did the standard housed animals. This is consistent with the findings of Thiessen et al (1962). They tested C3H mice from a standard group and from a group provided with toys, in a hole-in-wall test which is comparable to our emergence test. They found the mice from the enriched conditions to escape twice as fast as the mice from the standard conditions. Chamove (1989b) housed CLFP mice in cages differing in complexity and performed a box emergence test. The animals from the more complex cages escaped significantly faster than the control animals.

In this study the two groups of the BALB/c strain did not differ significantly from each other in emergence time. Although this might be strain specific it should be mentioned that the lighting during both the hole board and emergence test was bright. Henderson (1972) has stated that albino strains with non-pigmented eyes can be at a disadvantage in behavioural tests with bright background illumination. These mice have a poor visual discrimination and show an increased tendency toward freezing behaviour. We observed that in both groups of the BALB/c strain, some animals did not escape from the cage within 10 min. Different illumination levels during testing had little effect on the behaviour of a pigmented strain like the C57BL (Nagy et al 1970). Thus the different behavioural responses of BALB/c and C57BL mice may (partly) be caused by the differences in sensitivity to the illumination during these two tests.

Manosevitz (1970) and Manosevitz & Montemayor (1972) investigated the behaviour of respectively, random bred and inbred mice, housed under standard and enriched conditions. In both studies they found that animals from the enriched environment were more active than animals from the standard environment in an open field. In contrast Rose et al (1985) found lower activity scores in rats from an enriched environment in an open field test. These results are consistent with our observations during the first part of the open field test. We also found a lower

activity of the enriched housed BALB/c mice compared to the standard housed mice and the same tendency, although not statistically significant between the two groups of C57BL mice. No differences in defecation scores were found between enriched and standard housed animals in both strains. This is in concordance with the results of Manosevitz & Montemayor, but in contrast to previous findings of Manosevitz. According to Manosevitz & Montemayor this discrepancy in findings may be due to the different genotypes used in the studies. Furthermore, in our study mice were housed in the enriched environment after weaning, whereas in both Manosevitz studies mice were housed in enriched cages from birth on.

The C57BL mice housed in the enriched cage showed more locomotion and rearing and less immobility and freezing in the second part of the test. These behavioural changes may partly be a reaction to the sudden change in noise level and partly indicate that the enriched housed mice habituated more easily to the new situation in the second part of the test. Together with their behaviour in the cage emergence test and the hole board test it seemed that these animals were more reactive and alert than the animals from the standard environment. The BALB/c mice housed in the enriched environment differed for a number of behaviours from mice housed in the standard cage. Especially the differences in freezing and locomotion suggest a higher level of alertness or anxiety in these animals. Walsh & Cummins (1976) and Archer (1973) reviewed the validity of the open field test as a test for emotionality or anxiety. The test has been widely used but with various procedures and variables measured. Therefore the results must be interpreted with care. We used the test mainly to compare the behaviour of two differentially housed groups of mice and the results have shown that for a number of behavioural parameters, significant differences between the groups occurred.

With the two other behavioural tests it was also possible to discriminate between the different groups of mice, although an adaptation in lighting conditions when testing albino strains is advisable.

Overall, the results of the tests indicated that mice housed in the enriched environments were more dynamic in their reactions to novel situations than mice from the standard environments. They were more alert and seem to habituate faster as compared to their standard housed counterparts. However, the behavioural effects differed per strain. Manosevitz & Montemayor (1972) also have indicated that genetic factors are of considerable importance in mediating the effects of environmental enrichment.

The C57BL mice from the enriched environments consumed significantly less food than the standard housed animals. However there were no differences in body weight between the two groups. An explanation for this phenomenon might be a difference in thermoregulation of the animals. Chvédoﬀ et al (1980) studied food intake and body weight in groups of mice. They observed a decline in food

consumption with an increased cage density, but hardly any differences in body weight. Their explanation for this was that mice in groups sleep huddled together and by doing so reduce their overall body heat loss, compared to individually housed mice. In our study animals in the enriched cages had nesting material and shelters to sleep in providing them a good insulation. Although this was also the case for the BALB/c mice no difference in food consumption was observed between the two groups of this strain.

The observations in the animal room showed us that the mice in the enriched cage were able to structure their living environment by manipulating the enrichment objects. The animals were eager to use the enrichment provided and they made nests of the nesting material given. This is in concordance with the observations of Scharmann (1991). Fighting occurred mostly in the BALB/c mice. This strain is known to be more aggressive than the C57BL strain (Mondragón et al 1987).

From the present results it can be concluded that under the conditions of this study relatively simple enrichment of the environment has an influence on the animal's behaviour. The results indicated that C57BL mice housed in the enriched environment became more reactive and alert. In the BALB/c mice results may be interpreted as if the enriched environment lead to an increased level of anxiety.

The results of the present study suggest that the differential, strain specific behavioural response is an important factor to be taken into account when seeking the improvement of the animal's welfare by cage enrichment.